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New Neurotoxins From Venoms Of Aculeate Hymenoptera: A Contribution To The Knowledge Of Stinging Behaviour

Tom Piek

Department of Pharmacology, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

Abstract - Several Hymenoptera produce a venom which contains a neurotoxic component. Kinins, present in venoms of vespid, scoliid, tiphiid, multillid and formicid Hymenoptera, irreversibly block the transmission in the insect CNS presynaptically by depletion. Poneratoxin, a neurotoxic compound isolated from ant venom, affects the ion current in voltage-dependent channels in nerve and muscle fibres. Philanthotoxins block the neuromuscular transmission and the synaptic transmission in the CNS of insects. The results support the idea that entomophagous Aculeata sting their prey in the CNS and explain the effects of the sting on insects and mammals.

INTRODUCTION

Within the phylum Arthropoda several groups include well known producers of venoms: the spiders, the scorpions and the hymenopteran insects. In the first seventy years of this century a number of arthropod venoms has been described. A manual of information was presented in a volume on Arthropod Venoms edited by Sergio Bettini (1978). The biochemical, pharmacological and behavioural aspects of venoms produced by Hymenoptera were collected together by Piek (1986). Although the latter book provides the reader with extensive documentation of observations and research during the last 100 years on bee, wasp and ant venoms, several of the most interesting wasp and ant neurotoxins which have a relation to the stinging behaviour of these insects were not yet chemically characterized in the first half of the 1980's. It is the aim of this paper to review the several types of neurotoxic effects of solitary wasps and thus contribute to the understanding of stinging behaviour.

HYMENOPTERA AS NEUROTOXIN PRODUCERS

The earliest report of a neurotoxic effect on an insect by a solitary wasp may be in the Erh-ya yin t'u or Dictionary of Old Terms, of which the Sung illustrations may originate between 500 and 400 BC (Bodenheimer 1928). The idea, that "in seven days the worm stung by the wasp was transformed into the son of the wasp," conveys the notion that the prey was not killed but was otherwise incapacitated.

A principal question during the eighteenth and nineteenth centuries was whether prey of solitary aculeate wasps were killed or paralysed. Advocates of the killing idea were Réaumur (1742) and Dufour (1841). Sympathizers with the idea that prey of solitary wasps were not killed but paralysed were, for example, Bartram (1744, 1749) and Audouin (1839). It fell to Fabre (1855) to settle the conflict of views by electrically stimulating weevils, stung to a complete and irreversible immobility by the sphecid wasp *Cerceris tuberculata* (Villers) thus demonstrating that the prey was capable of movement and therefore in deep paralysis, not dead.

Solitary wasp venoms cause paralysis by neurotoxic action and we now know that neurotoxic principles are also found in bee venoms, social wasp venoms and ant venoms.

KININS FROM VESPID AND SCOLIID WASP VENOMS

Kinins are neurotoxic components of wasp and ant venoms, causing in the insect central nervous system a presynaptic block of the cholinergic transmission by means of an irreversible depletion of transmitter substance (acetylcholine), probably by means of a non-competitive inhibition of the presynaptic choline-uptake (Piek et al. 1987, 1990; Piek 1991).

Wasp kinins are polypeptides of 9-18 amino-acid residues containing a bradykinin-like sequence as part of the molecule. The bradykinin-like sequence is either identical to the vertebrate bradykinin, or differs in a single OH-group (prolin becomes hydroxyprolin, Hyp^3-bradykinin) or differs in a single CH_3-group (Thr^6-bradykinin). A bradykinin-like substance was discovered as a venom constituent of the wasp *Paravespula vulgaris* (L.) (Jacques and Schachter, 1954) and was called wasp kinin (Schachter and Tain 1954). Although the chemical characterization of this wasp kinin is still unknown, other social wasp kinins have been characterized chemically (for reviews see Nakajima 1986 and Piek 1991).

Despite the fact that wasp kinins have been known for more than 35 years, their neurotoxic actions had not been discovered before the demonstration of the presence of kinins in the venom of the solitary wasp *Megascolia flavifrons* (F.) (Piek et al. 1983, 1984b, Yasuhara et al. 1987). A point of interest is that this wasp stings larvae of the European rhinoceros beetle *Oryctes nasicornis* L. producing an irreversible paralysis, by successively penetrating the ventral side of all segments, which contain nerve ganglia (Piek et al. 1983). This scoliid wasp probably injects its venom into the nerve ganglia, a phenomenon which has commonly been observed for many solitary aculeate wasps (see also section on philanthotoxins). The idea that these wasps sting into the nerve ganglia prompted study of the effect of the venom and its fractions, and of purified or synthesized toxins, by means of microperfusion of an insect ganglion. This became the start of a series of experiments on the neurotoxic action of threonine-6-bradykinin (Thr^6BK), the most active kinin in the venom of *M. flavifrons* (Yasuhara et al. 1987) and of *Colpa interrupta* (F.) (Piek et al. 1990).

When a venom solution, prepared of isolated venom reservoirs of *M. flavifrons* was injected into the haemolymph of an *Oryctes nasicornis* larva, or when a female *M. flavifrons* was forced to sting the larva, no paralysis occured (Piek et al. 1983). However, the venom preparation was very active, as a blocker of synaptic transmission, when injected (by microperfusion) into the sixth abdominal ganglion of the cockroach, *Periplaneta americana* L. (Piek et al. 1987). It was shown that Thr^6BK irreversibly blocks synaptic transmission from the cercal nerve XI of the cockroach to a giant interneuron in the sixth abdominal ganglion (Hue and Piek 1988, 1989).

The presence of kinins as producers of neurotoxic insecticidal effects in the venoms of Vespidae, Scoliidae, Tiphiidae, Mutillidae and Formicidae might be resolved as a toxinological argument for the phylogenetic relationship of these groups (Piek 1991, 1992). However, part of this view is in conflict with other evidence (Brothers 1975).

PONERATOXIN, A NEUROTOXIC ANT VENOM COMPONENT

Compared to the information available for bee and wasp venoms, knowledge about neurotoxic ant venoms is limited. Nevertheless, ant venoms have provided new tools for the study of neurophysiology, such as piperidine alkaloids in fire ant venoms (review: Schmidt 1986) and poneratoxin (Piek et al. 1991 a,b).

Following envenomation of man by the ponerine ant *Paraponera clavata* (F.), Schmidt et al. (1984) reported uncontrollable trembling which was not caused by pain alone. The venom contains at least two fractions which block specifically neuronal signals in the insect central nervous system (CNS) (Piek et al. 1991a). One of the fractions of the crude venom was pharmacologically characterized as a kinin. Although the chemical structure of this ant kinin is still unknown, its pharmacological action is comparable to the wasp kinins described in the preceding section.

The second neurotoxic fraction proved to be the most potent blocker of CNS functions. It contained a very potent neurotoxic peptide of 25 amino acid residues, called poneratoxin (Piek et al. 1991a). This has been synthesized by Professor Terumi Nakajima (Tokyo). At concentrations varying from 10^{-8} to 10^{-6}M the synthetic poneratoxin (PoTX) blocks synaptic transmission in the insect CNS in a concentration-dependent way and it depolarizes giant interneurons. At comparable concentrations PoTX affects the electrical activity of isolated cockroach axons, as well as of isolated frog and rat skeletal muscle fibres. The explanation of these

actions is the finding that PoTX prolongs action potentials and thus induces slow automatic activity. This is a consequence of a slow sodium ion-current activation at unusual negative values of potential and due to slow deactivation (Piek et al. 1991b Duval et al. 1991, 1992). This explains the insecticidal action of a sting of the ant into an insect prey, and also the fibrillation of skeletal muscles of man (Schmidt et al. 1980) and insects (Piek et al. 1991b).

As a venom reservoir of a single ant specimen (*P. clavata*) may contain about 1 µg poneratoxin (Piek et al. 1991a), injection of its content may result, for small insects, in a final concentration of between 10^{-5} and 10^{-4}M. Even a tenth of the venom reservoir content injected into the CNS immediately and irreversibly blocks neuronal functions. For vertebrates about 30 stings of *Paraponera clavata* per kg can be lethal (LD_{50}: 33 stings/kg; Schmidt et al. 1980). If this results in about 30 µg of poneratoxin per kg, the final concentration of poneratoxin will be in the order of 10^{-8}-10^{-7}M, a level which is probably lethal. The uncontrollable trembling and unbearable pain caused by a sting by *P. clavata* is explained by the hyperactivity of the nervous network caused by prolongation of action potentials.

It is concluded that poneratoxin alone could explain the reversible neurotoxicity of the venom of *P. clavata*, but the kinin present in the venom, may contribute to the insecticidal action considerably by blocking irreversibly the synaptic neurotransmission.

PARALYSIS OF PREY BY PHILANTHOTOXINS

The two first examples of neurotoxins (kinins and poneratoxin) are polypeptides produced by vespoid Hymenoptera. Our knowledge of venoms of those wasps which belong to a quite different group, the Apoidea spheciformes (or sphecid wasps) is relatively poor. The venom and its constituent neurotoxins has been well described for only a single species, i.e. that of the sphecid wasp *Philanthus triangulum* (F.). The venom contains at least two different toxins that are antagonists for the glutamatergic neuromuscular transmission in insects (Piek and Spanjer 1986). The two toxins are chemically characterized as -ß-philanthotoxin (-ß-PTX) and -philanthotoxin (PTX-4.3.3) respectively (Karst et al. 1990, 1991, Karst and Piek, 1991). These philanthotoxins are polyamine-like structures that block both the insect neuromuscular transmission and the nicotinic synaptic transmission in the insect central nervous system (Hue and Piek 1989, Karst et al. 1990, 1991, Karst and Piek 1991).

STINGING BEHAVIOUR

In his *Souvenirs Entomologiques* Fabre (1879-1910) presented the view that solitary wasps sting their victims in the CNS. He observed prey having a concentrated CNS (buprestid beetles, weevils and some beetle larvae) to be stung once and observed that prey with a more diffuse CNS were stung more than once. Among his examples were *Sphex* spp. which sting crickets three times, once in each of the three main nerve ganglia, and *Ammophila* spp. which sting caterpillars in every segment containing a major nerve ganglion.

Based on observations over more than twenty years, Steiner (1986) tested in sphecid wasps six different hypotheses concerning sting number, location of sting, sting direction and other characteristics. Conceivably, the number, location and direction of successive stings could have been affected by (1) soft spots in the integument of the prey (Ferton's soft spots hypothesis; Steiner 1986), (2) body segmentation, (3) leg bases, (4) complete set of ganglia or (5) ganglia involved in locomotion and defence. The null hypothesis would be random stinging. Steiner (1986) concluded that although a wide spectrum of stinging methods could not easily be encapsulated in a single simple formula, the locomotor ganglia hypothesis of stinging is the best-fitting one for a number of aculeate wasps which prey on large or powerful insects. These wasps give at least one different sting for each clearly separate nerve centre involved in locomotion, attack, defence or resistance of the prey.

In a single case the localization in a cross section of the mesothorax of *Musca domestica* L. of radioactively labelled venom of the sphecid wasp *Mellinus arvensis* L. has been demonstrated (Piek 1978, see also Steiner 1986). The extensive documentation on stinging behaviour in relation to the

effects of stinging on prey (Steiner 1986, Piek and Spanjer 1986) suggests a general rule: entomophagous Aculeata sting into the CNS of the prey. The blocking action in the insect CNS of the venoms of *Philanthus triangulum* (Hue and Piek 1989) and of *Megascolia flavifrons* (Piek et al. 1987) also supports Steiner's conclusion.

Several unpublished pilot studies in our laboratories indicate that the venoms of sphecid wasps other than *P. triangulum* did not affect the insect neuromuscular transmission. We may speculate that the ability of *P. triangulum* to paralyse its prey (workers of the honeybee) by a peripheral effect on muscle contraction has been developed because of the dangerous defensive behaviour of the prey. When, for example, a female *P. triangulum* is brought together with ten honeybee workers, the wasp is sometimes killed by one of the last bees to be attacked. Therefore, it may be safer for the wasp to give a random sting, which quickly but reversibly incapacitates the bee. Subsequently a sting is given into the thoracic ganglion complex and the process is often completed by a sting into the suboesophageal ganglion. This complete stinging sequence results in a long-term paralysis.

A different, but to a certain degree comparable stinging behaviour has been described for *Ampulex compressa* F. and *Liris nigra* F. A sting in the thorax of the cockroach transiently paralyses the victim. During that short-lived immobility of the cockroach, the wasp stings carefully into the suboesophageal ganglion. Only this latter sting results in a delayed and irreversible change of behaviour of the cockroach (Steiner, 1986, Piek et al., 1984, 1989).

It might be clear that the explanation of the atypical venom composition of *P. triangulum* has to be supported by studies of venoms of other bee-hunting or wasp-hunting sphecid wasps. It may be a challenge to students of the biology of the solitary wasps to collect arguments in favour or against the above mentioned view.

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